

Neotype Designation for the Marine Flatworm, *Acanthozoon alderi* (Polycladida: Cotylea: Pseudocerotidae), from India with Comments on the Taxonomical Status of the Genus

Sudhanshu Dixit¹, Verónica N. Bulnes^{2,*}, and Chelladurai Raghunathan³

¹Centre for Marine Living Resources and Ecology, Kochi, India. E-mail: sid2130@gmail.com

²Laboratorio de Zoología de Invertebrados I. INBIOSUR-CONICET-UNS. San Juan 670. 8000 Bahía Blanca. Buenos Aires. Argentina

³Zoological Survey of India, M - Block, Alipore, Kolkata, India. E-mail: raghuks@rediffmail.com

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Sudhanshu Dixit, Verónica N. Bulnes, and Chelladurai Raghunathan (2018) *Acanthozoon alderi* is an ovoid, medium-sized pseudocerotid. Body margin ruffled; pseudotentacles black and pointed, with white tips. Dorsal surface covered with papillae, except for the cerebral region. Background colour light brown, with marbled blackish pattern, middorsal black band with white blotches; black submarginal band and marginal white rim. This species was described from Borneo; however, no type specimen was designated or deposited in any museum by the author. Many nomenclature problems and misidentification have been encountered with this species (it has been identified as *Acanthozoon* sp. in many instances). Thus, it is necessary to designate a neotype to solve the problems of doubtful and confusing identities and maintain nomenclature stability. The present paper details internal anatomy for the first time and makes important observations about the nominal status of the genus.

Key words: Marine flatworms, Andaman and Nicobar Islands, Cotylea, *Thysanozoon*, Dorsal papillae.

BACKGROUND

Polyclad biodiversity from Indian waters and related areas are still poorly described. The first contribution was made by Laidlaw (1902), who described seven new species from the Laccadives Archipelago, and only recently, with the newfound interest in the study of biodiversity, has that the number of reported or described polyclad species in this region increased to 52 (Bhadja 2010; Apte and Pitale 2011; Sreeraj and Raghunathan 2011 2013 and 2015; Dixit and Raghunathan 2013; Pitale et al. 2014; Sreeraj et al. 2015; Dixit et al. 2015 2017a b 2018a b).

The members of the order polycladida are dorsoventrally flattened marine free-living worms, with a much-ramified intestine and complex reproductive system. They are ubiquitous in marine environments, inhabiting shallow reefs (Newman and Cannon 2003), the water surface (Faubel 1984a), deep waters (Quiroga et al. 2006), and artificial aquaculture structures (Bahia 2015).

Polycladida taxonomy is based on the hermaphrodite reproductive anatomy and external morphology (eyespot arrangements, tentacles, and pharynx) (Hyman 1959; Faubel 1984b; Prudhoe 1985); the coloration pattern is also considered an important character in the identification at species

*Correspondence: E-mail: vebulnes@criba.edu.ar

level, especially between the members of the family Pseudocerotidae (Newman and Cannon 1995; Litvaitis et al. 2010; Bahia et al. 2017).

Acanthozoon alderi was originally described as *Thysanozoon alderi* by Collingwood (1876) and later transferred to the genus *Acanthozoon* by Faubel (1984b) based on the presence of well-developed dorsal papillae and a single male copulatory organ.

In the original contribution, Collingwood (1876) neither provided a catalogue number, nor named an institution where the holotype or the original plates were deposited. We contacted some different institutes and museums where we thought the type material may be deposited - including the Borneo Marine Research Institute (where Collingwood's specimen was collected), the Museum of Comparative Zoology of Harvard University, and the Natural History Museum in London - but found no evidence for the existence of such material. Nevertheless, we were able to locate some vouchers of *Acanthozoon alderi* in other collections not related to Collingwood's contribution: two specimens identified by Stummer-Traunfels (1895), one in the Museum of Zoology in Göttingen (collected by Brock in Ambon Island, 1885) and one in the Natural History Museum of Vienna (collected by Sarasin in Tricomalee), and a third voucher specimen, identified by Prudhoe in 1975, deposited in the Australian Museum in Sydney (collected by Coleman in the Lizard Islands, 1975). However, the poor condition of the exemplars (shrunken, broken, discoloured and curled) did not allowed us to unequivocally identify the material as *Acanthozoon alderi*.

We designate a neotype of *Acanthozoon alderi* and provide a detailed description of its external morphology and internal anatomy. We also extend and update its geographic distribution and the nominal status of the genus.

MATERIALS AND METHODS

The specimens were handpicked using a paintbrush from subtidal area off the Sound Island and Andaman and Nicobar Islands while SCUBA diving (Fig. 1) and photographed both in situ and after taking them to the laboratory while alive using a Cannon G-15 with housing. Specimens were fixed in frozen 10% formalin buffered with seawater (modified from Newman and Cannon 2003). The reproductive structures were dissected out to obtain 7 µm sagittal sections, stained

with haematoxylin and eosin. The taxonomic examination and measurements (length mm × width mm) of fixed specimens (total length and width, distance between mouth, gonopores and sucker) were made under a stereo microscope (Leica M 205 A). Figure 1 was prepared using ArcGIS software. All figures were edited using Adobe software (Photoshop and Illustrator) and PhotoScape. The type material is deposited in the National Zoological Collections at the Zoological Survey of India, Andaman and Nicobar Regional Centre (ZSI/ANRC).

RESULTS

Order: Polycladida Lang, 1884
Family: Pseudocerotidae Lang, 1884
Genus *Acanthozoon* Collingwood, 1876

Acanthozoon alderi (Collingwood, 1876)

Thysanozoon alderi Collingwood, 1876: 88 (Type locality: Labuan Island, Borneo); Stummer-Traunfels 1895: 706 (Ambon Island and Tricomalee, Sri Lanka); Prudhoe, 1975. Label of voucher W 8687 from the Australian Museum in Sydney (Lizard Island, Australia)

Material examined: Neotype. One specimen as serial sections (8 Slides), remainder of animal preserved in 70% ethanol, (ZSI/ANRC-16848), collected on 14th July 2013 at 10 m depth.

New type locality: Sound Island (12°56.167'N 092°58.113'E), Mayabunder, Andaman and Nicobar, India. The specimen was found crawling on rocky substratum in coral reef area.

Additional material: One specimen preserved whole in 70% alcohol (ZSI/ANRC-16849), collected on 16th December 2016 at depth of 12 m off Rutland Island (11°30.119'N 092°37.112'E), Andaman and Nicobar, India. The specimen was found crawling on rocky substratum in coral reef area.

Description: External morphology: Body ovoid, live specimen 5.5 mm long by 3 mm wide; fixed specimen 5 mm long by 4 mm wide (Fig. 2A). Background colour light brown, with a marbled blackish pattern, darker towards the margins and the middorsal strip. Middorsal black band with white blotches extending from right after the cerebral region to almost the distal margin without touching it. An inner black marginal band and an external white rim is present. Dorsal body surface and pseudotentacles covered with numerous papillae (3 to 5/mm²), except around the cerebral eye cluster (devoid of papillae), and

on the marginal white rim. The papillae are of various sizes, from 180 μm to 350 μm high (Fig. 3A), conical, light brown to white in their bases and yellow tips. Pseudotentacles prominent, pointed, well developed and black with white tips (Fig. 2A). Tentacular eyes not visible because of the epidermis pigmentation. Cerebral eyespots cluster horseshoe shaped with about 150 eyes. Pharynx large, ruffled, with 15 folds. Distance between male pore and sucker, 6.1 mm and between male and female gonopore, 1.3 mm (Fig. 2C).

Internal morphology: Dorsal body wall 43 to 120 μm high, epidermis cellular, ciliated, with presence of few rhabdites dorsally (Fig. 3A, B). The epidermal cells of varying heights, almost cuboidal on the surface of the body (30 μm), up to cylindrical on papillae tips (100 μm), loaded with a thin basophilic granular secretion. Big eosinophilic droplets in the distal cells of the papillae. Beneath the epidermis, there is a distinct basement membrane, somehow creased, and between the folds, the circular muscle fibres, followed inwards

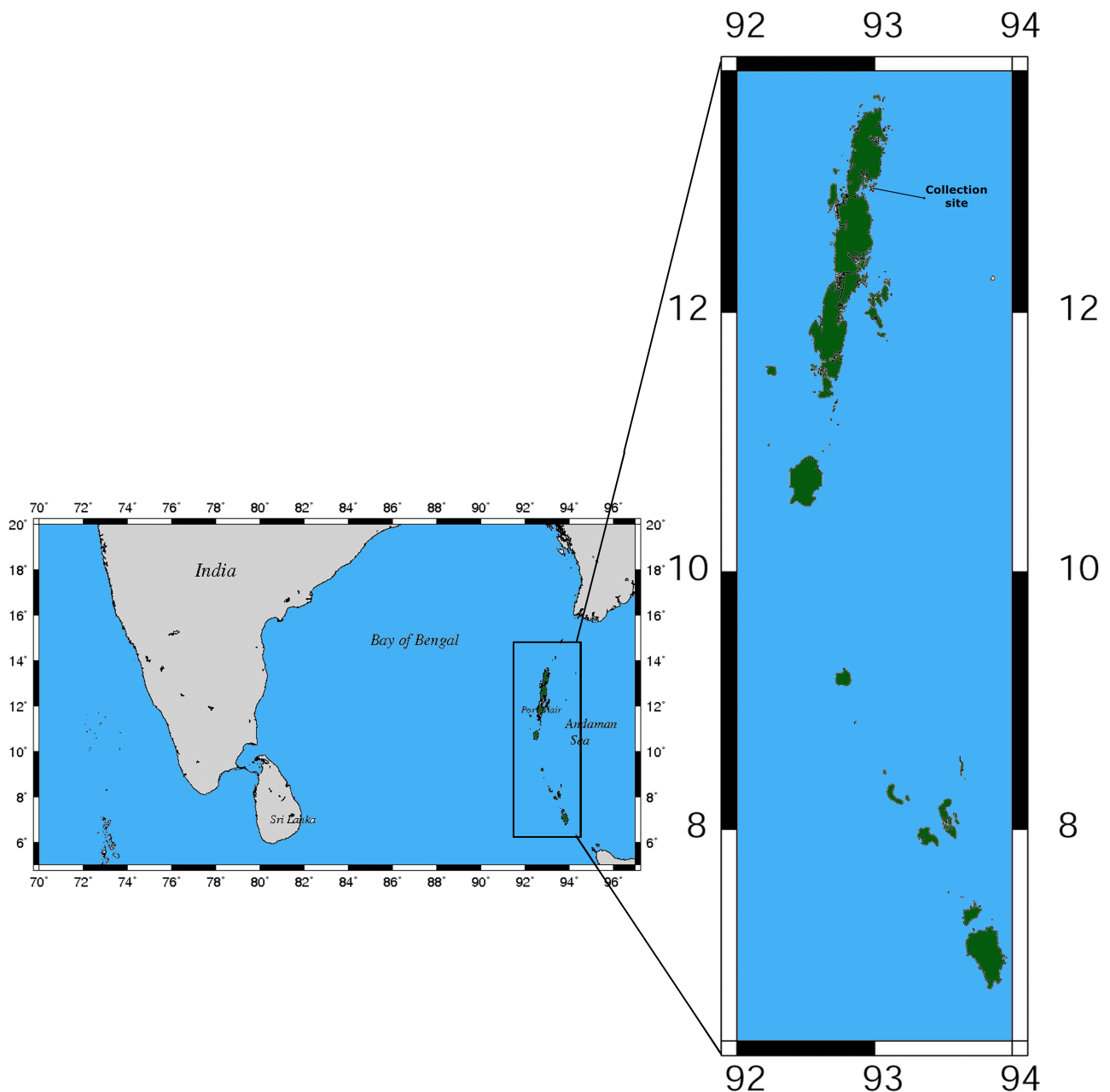


Fig. 1. Map of India and Andaman and Nicobar Islands (showing collection site). Author: Sudhanshu Dixit.

by a thin longitudinal muscle layer (Fig. 3A, B). Ventral body wall 40 to 54 μm high, epidermis ciliated, with scattered rhabdites. The epidermal cells are more homogenous in height than on dorsal surface. The basement membrane, the circular muscle layer and longitudinal muscle layer more developed than dorsal side. The parenchymatic tissue with numerous short muscular fibres arranged in all directions, being the dorso-ventral fibres, the longest. The intestine comprise one central branch and numerous

anastomosing lateral branches constituting a net (Fig. 3A, B). While over the pharynx and reproductive system, the branches are only dorsal, beyond this point, the branches are dorsal and ventral oriented. The main branch of the intestine is immersed in a well-developed layer of longitudinal muscle fibres. The muscular fibres are densely packed and sometimes the height of this layer exceeds the height of the inner intestinal tissue. In the secondary intestinal branches, the muscular layer is not apparent. Papilla without

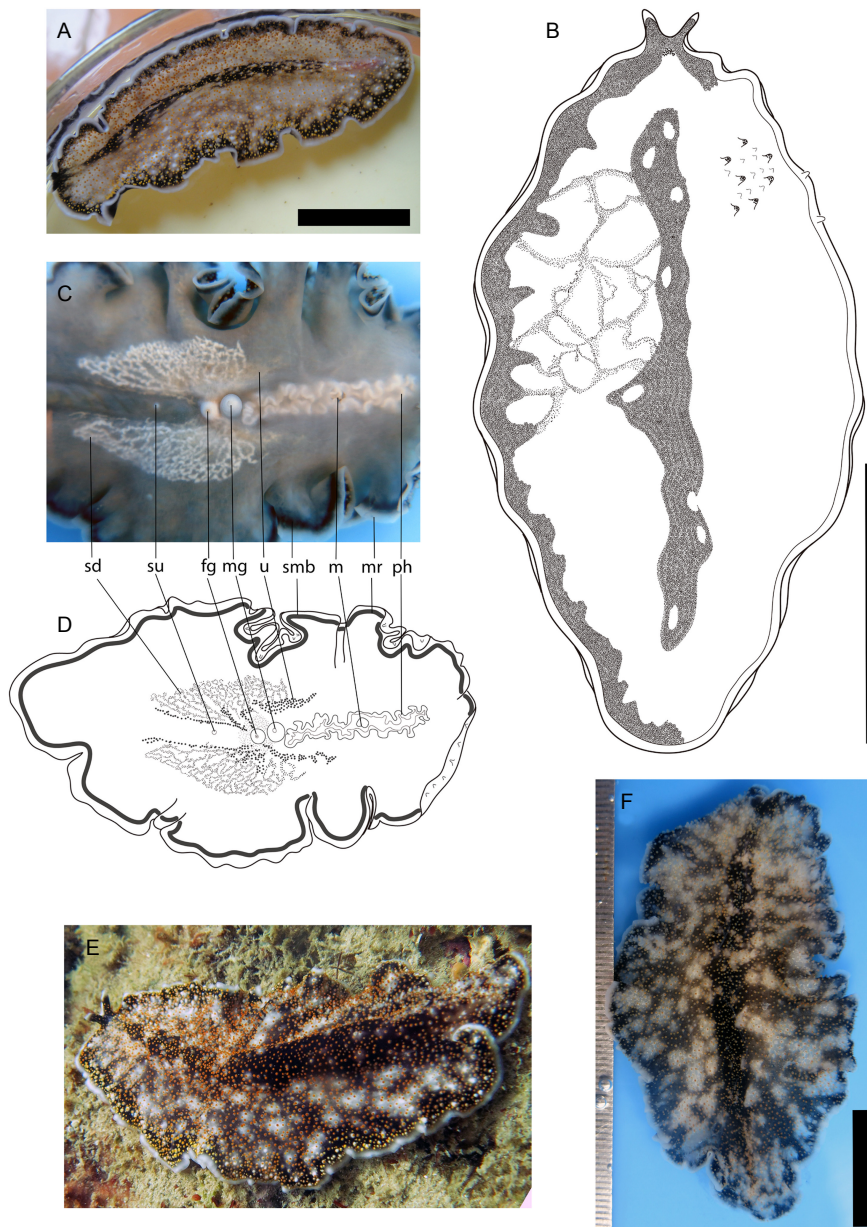


Fig. 2. *Acanthozoon alderi*. A, dorsal view of living specimen ZSI/ANRC-16848 (neotype). B, diagram of dorsal surface. C, ventral view of fixed specimen ZSI/ANRC-16849 (additional material). D, diagram of ventral view. E, dorsal view of living specimen ZSI/ANRC-16849 (additional material). F, dorsal view of fixed specimen ZSI/ANRC-16849 (additional material). fg, female gonopore; m, mouth; mg, male gonopore; mr, marginal rim; ph, pharynx; sd, seminal duct; smb, submarginal band; su, sucker. Scale bars: A, B and F, 20 mm.

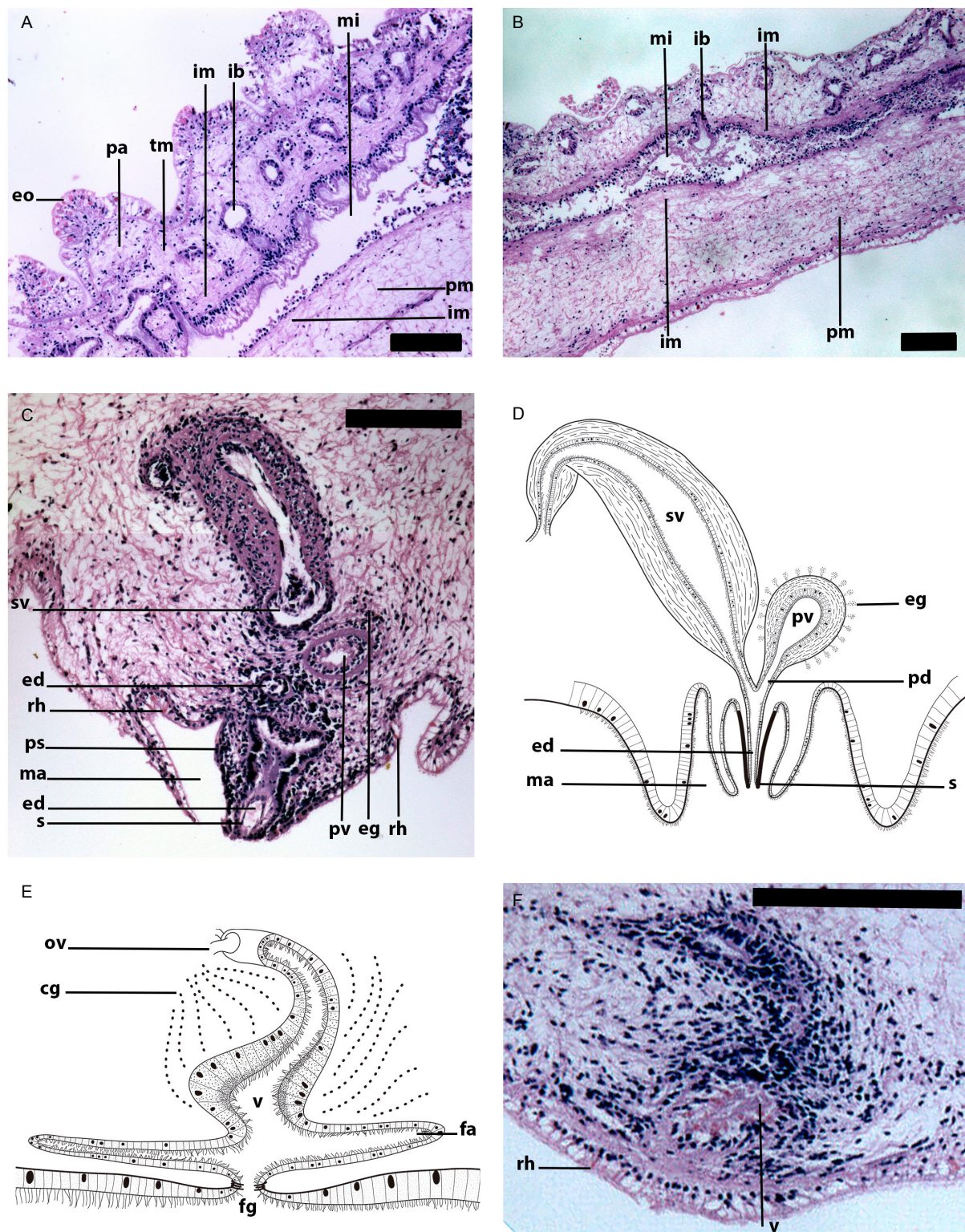


Fig. 3. *Acanthozoon alderi* (ZSI/ANRC-16848). A, sagittal section showing the dorsal papillae. B, sagittal section showing dorsal epidermis devoid of papillae. C, sagittal section of the male copulatory system. D, sagittal diagram of the male copulatory system. E, sagittal diagram of the female system. F, sagittal section of the female system. cg, cement glands; ed, ejaculatory duct; eg, extra-vesicular glands; eo, eosinophilous droplets; fa, female atrium; fg, female gonopore; ib, intestinal branch; im, intestinal muscle layer; ma, male atrium; mi, main intestine branch; ov, oviduct; pa, papilla; pm, parenchymal muscle fibres; ps, penis sheath; pv, prostatic vesicle; rh, rhabdites; sv, seminal vesicle; tm, transversal muscles; u, uteri; v, vagina. Scale bars = 200 μ m.

intestinal branches. The intestinal tissue is completely ciliated. The epithelium lining the main intestinal branch is higher than the epithelium of the lateral branches. In the first place, the cells are cylindrical, with basal nuclei, alternating highly vacuolated cells; with cells filled with eosinophilous granules. The inner epithelium of the lateral branches is lower, consisting in cuboidal cells, with central nuclei and rich in eosinophilous secretions. Uteri ramified, arranged dorsal to testes and seminal ducts, and extending from the beginning of the second quarter up to the end of the second quarter of the body. The male copulatory organs consist in a true seminal vesicle, a prostatic vesicle arranged free, and an armed penis (Fig. 3C, D). In their course, the seminal ducts combine forming a net, and join in a single duct on each side, just before entering the seminal vesicle from postero-dorsal. The seminal vesicle is elongate, 414 μm by 181 μm and is arranged more or less vertical dorsal to the prostatic vesicle. The prostatic vesicle is ovoid and small, 128 μm by 81 μm , immersed in a surrounding secretory tissue. The ejaculatory duct and the prostatic duct joins before entering the penis papilla. The penis papilla is armed with a short sclerotized tubular stylet, 150 μm long. The armed penis is surrounded by an epithelial fold, the penis sheath, housed in a well-developed male atrium. The epithelium of the penis sheath as well as of the male atrium completely ciliated. Scattered rhabdites opens in the male atrium and the ventral epidermis. The oviducts join before entering the vagina from postero-dorsal. The vagina oriented vertical (Fig. 3E, F). Proximally the inner epithelium is cuboidal, distal columnar, completely ciliated. The cement glands surround the female canal and opens distally in the vagina. The vagina opens to an extremely shallow female atrium, and to the female gonopore, provided with muscular ring. Scattered rhabdites in the atrium epithelium and the neighbouring ventral epidermis.

Additional observations: Some morphological variation was registered. The specimen, ZSI/ANRC-16849 was bigger (Fig. 2F), 7.7 \times 3.5 mm living and 7 \times 4 mm fixed. The colour pattern agrees with the above mentioned, but the middorsal band reach the hindermost part of the body, and the papillae were yellow towards the margin and orange to the centre of the body.

Distribution: Labuan Island, Borneo (former type locality); Ambon, Indonesia; Trincomalee, Sri Lanka, Lizard Island, Australia; Sound Island, Andaman and Nicobar, India (new type locality).

DISCUSSION

The following agree with the diagnosis of the genus given by Faubel (1984b) for the genus *Acanthozoon* Collingwood 1876: presence of an oval elongate form of the body; marginal tentacles with eye-spots, single cerebral eye-cluster; the dorsal surface scattered with pointed papillae; single male copulatory system, with seminal vesicle arranged medio-dorsally and armed penis; and the free prostatic vesicle oriented to the ejaculatory duct. Although the dorsal papillae are conical and not oblong, the ejaculatory duct is short and straight and not coiled, and we were not able to confirm that three branches of the uteri open on each side to the vagina, we believe that specimen studied belongs to the genus *Acanthozoon*.

Moreover, the following match the original description of *A. alderi* given by Collingwood (1876): the size of the thin body, with amply folded margin; dorsal background colour light brownish, with a darker marbled pattern, a white marginal rim, a sub-marginal black band, a mid-dorsal black band extending from the frontal three-quarters of body length; with numerous conical papillae, proximally white, with orange distal tips; and ventral surface whitish with a sub-marginal black band.

On the other hand, we observed a well-developed muscular layer consisting of longitudinal fibres surrounding the main intestine, absent in the secondary intestinal branches. This unique, distinct arrangement was never described in polyclads, and this organization of the muscular layers is probably responsible for the flexibility in the movement as well as stability of the translucent bodies.

The function of dorsal papillae of the Cotyleans are still unknown (Brusa et al. 2009), but there are some structural singularities that can be mentioned. The papillae being devoid of intestine, different from *Thysanozoon*, was also described for *Pseudoceros micropapillosum* Kato, 1934 and *Pseudoceros (Acanthozoon) hispidus* duBois Reymond Marcus, 1955, but the epidermal cells of the papillae are lower than on the rest of the dorsal epidermis. Hyman (1959) made a better account of the inner organization of the papillae of *Acanthozoon albopapillosum*. In her contribution, she described the differences in the height of the epidermal cells along the dorsal surface and the presence of apparent eosinophilous droplets in the distal part of the papillae, in addition to the absence of digestive epithelium. The only difference we

found in our material is the almost complete absence of rhabdites on the dorsal surface, and scattered on ventral surface and in the epithelium lining the male and female atrium. The papillae seem big in *Thysanozoon*, as high as the body or higher, with numerous rhabdites clusters, visible on the surface as small whitish spots, without eosinophilous droplets and with inner intestinal branches (Bulnes et al. 2011, Fig. 6D and 9C); the papillae seem smaller in *Acanthozoon*, barely higher than half of body height, devoid or with scattered rhabdites (when present only at papillae's bases), with eosinophilous droplets (coloured tips) and without intestinal branches.

Laidlaw (1902; p:306) described in *Acanthozoon plehni* the presence of a "curious plug-like of mass of tissue at the upper end of the diverticulum" and he suggested it may be connected with the excretory system. We were not able to find that kind of tissue in our *A. alderi* material.

CONCLUSIONS

The designation of a neotype

There is no record of the type specimen described by Collingwood (1876). Stummer-Traunfels (1895) provided a revision of the genus *Thysanozoon* based on the description of *Thysanozoon brochii*. Additionally, he described some specimens of *Acanthozoon alderi* collected in Ambon (Indonesia) and Trincomalee (Sri Lanka) (as *Thysanozoon alderi*). In his contribution, he registered some morphological variability in the size and colour pattern regarding Collingwood's description. However, the author did not provide a detailed description of the microanatomy of *T. alderi* and only compared a few characters with *T. brochii*. On this matter, the author only pointed out the presence of a simple penis and the papillae devoid of intestine extensions, different from the *Thysanozoon brochii* (Stummer-Traunfels, 1895; p. 706). Our material agrees with the description of the dorsal papillae devoid of intestinal extensions, with an armed penis, surrounded by a well-developed penis sheath and housed in an ample male atrium. The state of the specimen from Ambon deposited in the Museum of Zoology in Gottingen (ZMUG 26961) did not deliver much information. The specimen from Sri Lanka deposited in the Natural History Museum of Vienna (Catalogue number EV-2953-

om-*Thysanozoon alderi*-1 to 24) could not be borrowed. Nevertheless, we were able to obtain several photos of the slides and realized that most of the differential staining is lost. In 1975, Prudhoe identified a specimen of *T. alderi*, collected by N. Coleman from Lizard Island, Australia, which is deposited in the Australian Museum in Sydney (Catalogue number W.8687, Fig. 4E), but no published records of this specimen are available in any kind of report or publications. No histological studies were done and the actual state of preservation did not allow us to obtain more information from this specimen.

The general state of the museum material from Gottingen, Vienna and Sydney allowed us to complete the description of this species based on our own material. Thus, we believe that it is best to designate a neotype based on a specimen deposited at ZSI/ANRC, which is a national repository and publically accessible (International Code of Zoological Nomenclature 1999, article. 75.3.7). The designation of a new type locality is a mandatory act after the article 76.3 (ICZN 1999) and we fulfil this act in order to achieve stability and sense in the scientific naming of animals. *Acanthozoon alderi* is reported in the Indo-Pacific region from Borneo (Labuan Island); Sri Lanka (Trincomalee) and Australia (Lizard Island). Although the present type locality is in Andaman Islands and not Labuan Island, the new type locality is within the distribution range of this species in the Indo-Pacific region, thus designating a new type locality will not create any confusion regarding its distribution in a geographical realm (Indo-Pacific). As this species was very briefly described and often misidentified, this designation will clarify its taxonomic status (ICZN 1999, article 75.3.1). This neotype replaces lost or undesignated holotype after an extensive search for the type material (ICZN 1999: article 75.3.4; see background section for steps taken to trace type material) and is in accordance with the original description (ICZN 1999: article 75.3.5).

Extending the known distribution of *Acanthozoon alderi*

After the original description made by Collingwood (1876), only Stummer-Traunfels (1895) and Prudhoe (Fig. 4E) were able to identify *Acanthozoon alderi*. In the following years, many researchers and photographers have published some of their work on the internet but identified the specimens as *Acanthozoon* sp. We believe the

examples illustrated in the following literature and links belong to *Acanthozoon alderi*.

Acanthozoon sp. 2 from Papua, New Guinea, Indonesia, Marshall Islands, Thailand, Philippines, Japan and Maldives (Newman and Cannon, 2003, photo from the front page, title page, foreword, introduction, and p. 29, 47, 52, 71; and Newman and Cannon, 2005); *Acanthozoon* sp. from the Philippines ([https://www.rzuser.uni-](https://www.rzuser.uni-heidelberg.de/~bu6/flat0136.html)

[heidelberg.de/~bu6/flat0136.html](https://www.rzuser.uni-heidelberg.de/~bu6/flat0136.html)); *Acanthozoon* sp. from Singapore (<http://www.wildsingapore.com/wildfacts/worm/polycladida/acanthozoon.htm>); *Acanthozoon* sp.2 from Indonesia, West Papua (<http://www.nudibranch.org/Flatworms/indonesia/html/acanthozoon-sp2-01.html>), North East Sulawesi (<http://www.nudibranch.org/Flatworms/indonesia/html/acanthozoon-sp2-02.html>), Flores Island, Indonesia (<http://www.nudibranch.org/>



Fig. 4. A and B, voucher specimen of *Thysanozoon alderi* from the Museum of Zoology in Gottingen (ZMUG 26961). C, voucher specimen of *Thysanozoon alderi* from the Natural History Museum of Vienna (EV-2953-om-*Thysanozoon alderi*-1-24). D and E, voucher specimen and label of *Thysanozoon alderi* from the Australian Museum in Sydney (W.8687).

Flatworms/indonesia/html/acanthozoon-sp2-03.html).

In light of the new evidence presented in this article, we propose extending the distribution of the species from Borneo to India.

Nomenclatural notes on the genus *Acanthozoon*

Collingwood (1876) created the new genus *Acanthozoon* based on two specimens from Ceylon described by Kelaart (1858) in a contribution that contained a brief description of 19 invertebrate species, without plates, but with enough information to make the species names available (ICZN 1999). Years later, Collingwood (1876) obtained Kelaart's figures and, since Kelaart's work was not yet acknowledged, he decided to include the species described by Kelaart in his own text. The history of how Collingwood got access to Kelaart's figures was documented by Collingwood himself (1876; p. 84) and later by Pethiyagoda and Manamendra-Arachchi (1997). Collingwood wrote that the diagnosis of the Ceylon's species was made based on "Dr. Kelaart's descriptions of his figures" and assigned the authorship to Kelaart. In addition, Collingwood (1876; p.85) stated, "I have ventured to constitute a new genus of two of the Ceylon species from a character which does not appear to be shared with any of those previously described. However, as all the Ceylon species have already been named by their collector and delineator, I shall of course adopt his specific terms, only inserting them in what appears to me to be their proper generic group". Two species, *Acanthozoon armatum* (Kelaart, 1858) and *Acanthozoon papilio* (Kelaart, 1858), were included in the new genus *Acanthozoon*. Since the genus *Acanthozoon* is neuter, *A. armatum* was correctly named. On the other hand, there is no explanation for his decision regarding the second species and the specific name *papilio* is considered an incorrect spelling, and the valid name is *Acanthozoon papilionis* (Kelaart, 1858). Lang (1884) transferred the *Acanthozoon* species to *Thysanozoon* Grube, 1840, and many authors followed that decision i.e. and Stummer-Traunfels (1895) and Prudhoe (1985). Years later, Marcus (1950), after describing the dorsal structures of a new species of *Pseudoceros*, recommended the revalidation of the genus *Acanthozoon* (Marcus, 1950; p.582). Hyman (1959) followed Marcus' (1950) advice and in her contribution she gave a new definition to the genus *Acanthozoon* (Hyman, 1959; p.581) and

designated *Acanthozoon armatus* (Kelaart, 1858) (should have written *Planaria armata* Kelaart, 1858) as type species of the genus (articles 69.1 ICZN), before describing two new *Acanthozoon* species. Faubel (1984b) extended the diagnosis of the genus and designated a new type species after he gave the taxonomic status of *incertae cedis* to *A. armatus*. Since then, most authors like Tyler and collaborators (2006 - 2016), have adopted Faubel's decision. Nevertheless, and since we could not find a valid substantiation to this action in the text, we think that Faubel made an involuntary mistake (ICZN 1999, article 70.2) and confused the nominal with the taxonomical status of the genus. The fact that Faubel (1984b, p. 207) considered it not possible to recognize *A. papilionis* and *A. armatum* is not a valid argument for changing the type species of a genus (ICZN 1999, article 61.1.3). Thus, the valid nominal status of the genus is: Genus *Acanthozoon* Collingwood 1876; type species *Planaria armata* Kelaart, 1858.

List of abbreviations

cg, cement glands.
ed, ejaculatory duct.
eg, extra-vesicular glands.
eo, eosinophilous droplets.
fa, female atrium.
fg, female gonopore.
ib, intestinal branch.
im, intestinal muscle layer.
m, mouth.
ma, male atrium.
mg, male gonopore.
mi, main intestine branch.
mr, marginal rim.
ov, oviduct.
pa, papilla.
ph, pharynx.
pm, parenchymal muscle fibres.
ps, penis sheath.
pv, prostatic vesicle.
rh, rhabdites.
sd, seminal duct.
smb, submarginal band.
su, sucker.
sv, seminal vesicle.
tm, transversal muscles.
u, uteri.
v, vagina.

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Authors' contributions: Sudhanshu Dixit is responsible for the collection and preparation of the specimens. He carried out the photographic register and the histological processing, contacted some of the above-mentioned scientific institutions and prepared the manuscript. Verónica N. Bulnes prepared the line diagrams and figures, contacted some of the above-mentioned scientific institutions, and prepared the manuscript. C. Raghunathan is responsible for the initial corrections and editorial inputs to the manuscript.

Competing interests: Sundhansu Dixit, Verónica N. Bulnes and C. Raghunathan declare that they have no conflict of interest.

Availability of data and materials: The materials used in this manuscript are deposited in reference institution detailed in the corresponding sections in the text.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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